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Photo-Signal Transduction in Motile Cilia <u>Blepharisma</u>

FINAL PROGRESS REPORT

Pill-Soon Song

U.S. ARMY RESEARCH OFFICE

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Specific aims of the study were to elucidate: (a) the chemical structures of light-sensor molecules blepharismin and stentorin, and (b) their mechanisms of action in light-sensory signal processing events, namely, detection of sudden changes in light intensity and light-avoiding behaviors of the single cell ciliate Blempharisma japonicum and its closely related organism Stentor coeruleus.

During the period of this grant, two significant results have been achieved. For the first time, we have been able to isolate and purify the photosensor-bound protein, stentorin, with molecular weight of 5,000. This light-sensor protein retains the functional characteristics of native stentorin protein complex of more than half a million molecular weight located within the pigment granules of *Stentor coeruleus*. In addition, we have been able to determine a tentative chemical structure for the light sensor chromophore of *Blepharisma japonicum*. It appears that the chemical structure of blepharismin is distinctly different from that of stentorin. Both stentorin and blepharismin add to the exclusive list of only a limited number (four to five) of light sensor molecules in nature.

Light signals perceived by the single cell ciliates are transduced by transducin-like G-proteins, as suggested by our study of the effects of various G-protein activators and inhibitors on the photo-sensory responses of both organisms. To characterize the G-proteins in the ciliate cells, Phun Bum Park and Elisabetta Bini supported by this grant partially cloned and sequenced Blepharisma and Stentor G-proteins. Photo-activation of G-proteins appears to be coupled to a cGMP-dependent phosphodiesterase. If this result is further confirmed by directly isolating and/or cloning the latter and establishing its light activation via G-proteins, the single cell photo-signal transduction system will provide an interesting comparison to the visual excitation system in mammals.

- (1) LIST OF MANUSCRIPTS published under ARO sponsorship during this grant period (includes publications arising from both DAAL03-92-G-0356 and 28748-LS-SM with identical project title):
- 1. N. Tao, M. Orlando, J.-S. Hyon, M. Gross and Pill-Soon Song, A New Photoreceptor Molecule from *Stentor coeruleus*. J. Am. Chem. Soc. <u>115</u>, 2526-2528 (1993).
- 2. **Pill-Soon Song**, Structure and Function of the Ciliate Photoreceptors. In: <u>Frontiers of Photobiology</u> (Edited by A. Shima, M. Ichihashi, Y. Fujiwara and H. Takebe), International Congress Series 1021. Excerpta Medica, Amsterdam and New York, 1993. pp. 153-157.
- 3. I. Yamazaki, T. Yamazaki, Y. Nishimura, R. Dai and **Pill-Soon Song**, Time-Resolved Fluorescence Spectroscopy and Photolysis of the Photoreceptor Blepharismin. <u>Biochim. Biophys. Acta</u> <u>1143</u>, 319-326 (1993).
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- 5. S. Fabczak, H. Fabczak, N. Tao and **Pill-Soon Song**, Photosensory Transduction in Ciliates. I. An Electrophysiological Analysis of the Photophobic Response in *Stentor coeruleus*. <u>Photochem. Photobiol.</u>, **57**, 696-701 (1993).

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- 12. N. Tao and Pill-Soon Song, New Light Sensor Molecules of Single-Cell Ciliates. <u>Smart Structure and Materials</u>: SPIE Proceedings, Vol. <u>2189</u>, 238-248 (1994)
- 13. S. Fabczak, H. Fabczak and **Pill-Soon Song**, Ca²⁺ Ions Mediate the Photophobic Response in *Blepharisma* and *Stentor*, <u>Acta Protozoologica</u>, <u>33</u>, 93-100 (1994).
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